

IDPAS # 361 B

Weekly micronutrient supplementation to build iron stores in female Indonesian adolescents¹⁻³

Imelda Angeles-Agdeppa, Werner Schultink, Soemilah Sastroamidjojo, Rainer Gross, and Darwin Karyadi

ABSTRACT Different supplementation schemes to build iron stores in female Indonesian adolescents were investigated. Subjects were 273 high-school girls allocated randomly to four treatment groups. During a 3-mo period one group received 60 mg Fe, 750 μ g retinol, 250 μ g folic acid, and 60 mg vitamin C per day; a second group received 60 mg Fe, 6000 μ g retinol, 500 mg folic acid, and 60 mg vitamin C once a week; a third group received 120 mg Fe and the same amount of the other three micronutrients as the second group once a week; and a fourth group received only placebos. All subjects were dewormed and supplement allocation was double blind. Blood samples were collected at baseline, after 2 and 3 mo of supplementation, and 6 mo after the last supplement. After 2 mo of supplementation, groups supplemented weekly and daily showed similar significant improvements ($P < 0.001$) in hemoglobin and retinol concentrations, and supplementation for 3 instead of 2 mo did not significantly increase these two indicators. After 3 mo, the increase in ferritin was $\approx 27 \mu\text{g/L}$ in the daily and 14–15 $\mu\text{g/L}$ in the weekly groups ($P < 0.001$), the latter having a final concentration of 42–45 $\mu\text{g/L}$. At 6 mo postsupplementation there were no significant differences among daily and weekly groups, but the ferritin concentration was still ≈ 10 –12- $\mu\text{g/L}$ higher ($P < 0.001$) than in the placebo group. The group supplemented weekly with 60 mg Fe complained less about side effects than the other supplemented groups ($P < 0.05$). Weekly supplementation with 60 mg Fe and 6000 μg retinol for 3 mo was optimal for improving the iron status of the adolescents for ≈ 9 mo. *Am J Clin Nutr* 1997;66:177–83.

KEY WORDS Adolescents, iron deficiency, vitamin A deficiency, weekly supplementation, micronutrients, Indonesia, females

INTRODUCTION

Iron deficiency anemia is the most prevalent nutritional disorder worldwide (1). Pregnant women are at special risk and the prevalence of anemia for this population in the developing countries of Southeast Asia is as high as 60–70% (2). Because iron deficiency anemia is associated with poor pregnancy outcome (3, 4), many countries have started programs that attempt to increase iron intake during pregnancy by distributing iron tablets to pregnant women (5). Despite these programs, the prevalence of iron deficiency has not decreased in the past decade (1, 5). The low effectiveness of the iron-supplementation programs is considered to be mainly due to inefficient

health service delivery and to low compliance of the pregnant women with tablet intake (5–7); efforts should be undertaken to improve both of these factors. However, to decrease the prevalence of anemia during pregnancy it may not be sufficient to only focus on an improvement in the effectiveness of the existing iron-supplementation programs. Other factors, such as the timing of supplementation and possible deficiencies of other micronutrients, should also be considered.

Many women in developing countries are already anemic or have low iron stores before they get pregnant (6). These women will probably also be or become anemic soon after the iron requirements increase toward the end of the first trimester of pregnancy, and iron supplementation, which is normally provided during the later pregnancy stages, is then too late. Another factor is the vitamin A status of the women because vitamin A influences iron metabolism (8). Even when iron supplements are taken, any improvement in iron status may be limited when vitamin A status is low (9). A decrease in the prevalence of anemia during pregnancy could therefore be reached through an improvement in iron and vitamin A status of women before they get pregnant.

Adolescent girls would be an important target group because their iron requirements are relatively high, they will be future mothers, and they can often be reached relatively easily through schools (10). However, considering the experience with the supplementation program for pregnant women in terms of tablet delivery through the health care system and the low compliance with intake, it would probably be expensive and ineffective to supplement large groups of adolescent girls with iron and vitamin A by using the same approach as for pregnant women. A cheaper alternative (11) could be supplementation on a weekly basis because recent studies have shown that the effects of weekly iron supplementation are similar to those of daily supplementation among children (12, 13) and nonpregnant (14) and pregnant (15) women. Currently, no

¹ From The Regional South East Asian Ministries of Education Organization Center for Community Nutrition at the University of Indonesia, Jakarta, Indonesia, and the Deutsche Gesellschaft für Technische Zusammenarbeit GmbH, Eschborn, Germany.

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³ Address reprint requests to W Schultink, PO Box 3852, 10038 Jakarta, Indonesia. E-mail: gtzseame@indo.net.id.

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large-scale supplementation programs for adolescent girls exist, and whether weekly supplementation would benefit the girls immediately and in a possible future pregnancy would need to be investigated. It was the aim of this study to compare different frequencies and doses of supplements with multiple micronutrients among adolescent girls to investigate the effect of different durations of supplementation and the moderately long-term (9 mo) effect on iron stores.

SUBJECTS AND METHODS

The study was carried out in 1995 in East Jakarta. Subjects were girls who were enrolled in a governmental senior high school. A preliminary survey among 805 girls in the school showed that 21.1% of the girls were anemic with a hemoglobin concentration < 120 g/L (by finger-prick blood test). This prevalence was considered to be high enough to justify a blanket supplementation approach. Sample size calculation showed that with 60 subjects per group, a between-group difference in hemoglobin of 5.4 g/L and in retinol of 0.12 $\mu\text{mol/L}$ would be significant with an α of 0.05 and a power of 0.90. These differences were considered to be biologically significant. Allowing for a high dropout rate, we decided to include 360 girls and used presence of regular menstruation as a selection criterion. All 773 menopausal girls who were aged 14–18 y were eligible. Finally, 363 girls were randomly selected among the eligible girls, who, after selection, were allocated randomly to one of four treatment groups. Both the subjects and their parents gave written consent to participate in the study.

Information on the content of the supplements is provided in Table 1. The supplements were specially made for the study (Kimia Farma, Jakarta, Indonesia). Besides iron and vitamin A, the supplements also contained vitamin C to improve iron absorption and folate to improve folate status. One group received a supplement on every school day of the week (Monday-Friday); two other groups received a supplement only on Friday (Muslim prayer day) and placebos on the other school days; and the last (fourth) group received placebos on every school day. Supplements and placebos had the same red color and shape, and were not distinguishable by sight. The supplements were taken during a break in the teaching schedule and no strict prescription was given regarding tablet ingestion with

or without meals or snacks. All subjects were dewormed at the start of the study with a single 500-mg dose of mebendazole because 34% of a subsample of 104 subjects were found to be infested with *Trichuris trichiura* (16). The study was conducted in a randomized, doubly masked manner.

It was our intention to provide the supplements under supervised conditions during the whole 12-wk period. However, the last 4 wk of supplementation fell in the Muslim fasting month, and the girls had off both weeks 9 and 12 of the supplementation period. Therefore, supplementation was supervised during the first 8 wk, and during weeks 9–12 the supplements were provided on a take-home basis after the girls had received careful instruction on tablet intake. Although instructions were given, tablet intake during weeks 9–12 of supplementation was checked by asking the subjects at the end of the supplementation period whether they had taken all tablets. In addition, the plastic bags in which the tablets were provided were retrieved from all subjects and the remaining tablets were counted.

Specific side effects were investigated by asking the girls, on the Friday of week 11, whether they ever experienced an effect related to the supplement during weeks 10 and 11 of the supplementation period. The occurrence of side effects was reported as the percentage of subjects who experienced a certain effect at least once.

At baseline and after 8 and 12 wk of supplementation, nonfasting venous blood samples were collected at the school between 0900 and 1130. Blood collection fell outside the Muslim fasting month. To determine the longer-term effect of the supplementation, blood samples were again collected 6 mo after the end of the 12-wk supplementation period (9 mo after baseline). Blood was collected in two tubes: one tube containing heparin for the analysis of ferritin and retinol (5 mL blood), and one tube containing EDTA for the analysis of hemoglobin and white blood cell counts (3 mL blood). Blood was transported to the laboratory in a cool box and analysis of hemoglobin was done within 4 h after blood collection. Plasma was obtained by centrifugation for 10 min at $3000 \times g$ and 25°C , and was stored at -20°C until analyzed. Blood and plasma for retinol analysis were kept in the dark as much as possible.

Hemoglobin was analyzed by using a Coulter counter (Coulter MAXM; Coulter Electronics Limited, Luton, United Kingdom). A duplicate analysis was done in 40 samples. Variability based on these duplicate measurements was 0.99 g/L (17). Ferritin was analyzed by using the enzyme immunoassay method (17). Duplicate analyses were done on 12% of the samples. Variability based on these duplicate ferritin measurements was 0.246 $\mu\text{g/L}$ (17). Retinol was analyzed by using HPLC (18) in a darkened room. No duplicate analysis was done for retinol.

The study protocol was approved by the ethical review committee of the University of Indonesia and was in agreement with the international ethical guidelines for epidemiologic studies (19).

Statistical analysis

A one-sample Kolmogorov-Smirnov test was used to investigate whether the concentrations of hemoglobin, ferritin, and retinol resembled a normal distribution. Ferritin was not normally distributed but became so after a square-root transformation. Differences in concentrations between groups at baseline, 8 wk, 12 wk, and 6 mo after the end of supplementation

TABLE 1
Composition of supplements and frequency of intake

| Treatment group | Content of supplements | | | |
|--------------------------------|------------------------|---------------|-----------|--------|
| | Iron | Retinol | Vitamin C | Folate |
| | mg | μg | mg | mg |
| Daily ¹ | 60 | 750 | 60 | 250 |
| Weekly | | | | |
| Low iron content ² | 60 | 6000 | 60 | 500 |
| High iron content ² | 120 | 6000 | 60 | 500 |
| Placebo ³ | 0 | 0 | 0 | 0 |

¹ Micronutrient supplement administered every school day from Monday to Friday.

² Micronutrient supplement administered on Friday, with a placebo from Monday to Thursday.

³ Placebo administered every school day from Monday to Friday.

were tested with analysis of variance. Differences in treatment effect between the four groups and between the three supplemented groups were investigated by using the multivariate analysis (MANOVA) repeated-measures design of SPSS/PC+ (SPSS Inc, Chicago) (20) with the supplement types as a between-subjects factor and treatment effect (baseline compared with 8 wk, baseline compared with 12 wk, 8 wk compared with 12 wk) as a within-subjects factor. Between-group differences in treatment effect would be indicated by a significant interaction between treatment effect and treatment type. Initial hemoglobin (in three groups: < 120, 120–130, and > 130 g/L) and retinol (in three groups: < 0.70, 0.70–1.05, and > 1.05 $\mu\text{mol/L}$) concentrations were included in the analysis as between-subjects factors to correct for their possible confounding influence on the changes in hemoglobin and retinol. Differences in prevalence were tested with a chi-square test.

RESULTS

Between baseline and 12 wk, a complete data set was obtained for 273 subjects. Selected characteristics of the group of subjects at baseline ($n = 363$) and of the subjects who completed the study ($n = 273$) are shown in Table 2.

The number of dropouts from the daily, weekly with 60 mg Fe, weekly with 120 mg Fe, and placebo group were 28, 20, 27, and 15 respectively. The dropouts were not significantly different in age, weight, height, length of menstruation, or initial hemoglobin concentration (varied from 126 to 129 g/L). These values for the dropouts were also similar to the values of the remaining subjects (Table 2). Common reasons for dropping out were refusal to undergo blood taking, absence from class on the day of blood taking, and refusal to take tablets for the duration of 12 wk. Prevalence of low iron and vitamin A status is also presented in Table 2. At baseline, 30.4% had a ferritin concentration < 15 $\mu\text{g/L}$ and 20.1% had a ferritin concentration < 12 $\mu\text{g/L}$. Three subjects (1.1%) had a retinol concentration < 0.35 $\mu\text{mol/L}$. Of those subjects who had initial hemoglobin concentrations < 120 g/L, 68.1% had a ferritin concentration < 15 $\mu\text{g/L}$, and 57.4% had a retinol concentration < 0.70 $\mu\text{mol/L}$. There were low but significant correlations at baseline between concentrations of hemoglobin and

ferritin ($r = 0.37$, $P = 0.001$), and between hemoglobin and retinol ($r = 0.22$, $P = 0.001$). Retinol concentrations were also correlated with ferritin ($r = 0.20$, $P = 0.01$).

There were no significant differences in initial hemoglobin, retinol, or ferritin concentrations between the four groups (Table 3). After 8 wk of supplementation there were significant within-group increases ($P < 0.001$) in hemoglobin, ferritin, and retinol in the weekly and daily supplemented groups. In the placebo group the hemoglobin and ferritin concentration decreased ($P < 0.02$) whereas the retinol concentration increased compared with baseline ($P < 0.001$). The additional 4 wk of supplementation did not give the same uniform result for the groups supplemented weekly and daily. After 12 wk of supplementation, the hemoglobin concentration in the groups supplemented weekly and daily had decreased significantly compared with the values at 8 wk ($P < 0.001$) but was still higher than the baseline values ($P < 0.001$). The hemoglobin concentration of the placebo group also decreased significantly ($P < 0.001$). The ferritin concentration of the groups supplemented weekly was significantly higher at 12 wk than at 8 wk ($P < 0.001$) whereas for the group supplemented group daily there was no further significant increase. Compared with the value at 8 wk, the retinol concentration of the group supplemented weekly with 120 mg Fe had increased significantly ($P < 0.001$), and the retinol concentration in the placebo group had decreased ($P < 0.001$).

Although at baseline the concentration of hemoglobin and retinol was correlated, the changes in hemoglobin were not correlated with the changes in retinol for the three supplemented groups ($P = 0.63$). Between-group differences in treatment effect were tested separately for 8 and 12 wk of supplementation by investigating the changes compared with baseline. The concentrations at baseline were associated with the respective changes in hemoglobin ($r = -0.58$, $P < 0.001$) and retinol ($r = -0.62$, $P < 0.001$) concentration in the three micronutrient-supplemented groups. Therefore, the initial concentrations were included as confounding factors in the analysis of between-group differences in treatment effect for hemoglobin and retinol. Changes compared with baseline for all three hematologic indexes in the groups supplemented daily and weekly differed significantly from those in the placebo group at both 8 ($P < 0.001$) and 12 wk ($P < 0.001$) (Table 3). There was no significant difference in treatment effect on hemoglobin and retinol concentration between the groups supplemented weekly and daily at 8 or 12 wk. The increase in ferritin concentration in the group supplemented daily was greater than that for the groups supplemented weekly at both 8 ($P < 0.001$) and 12 ($P < 0.001$) wk.

Prevalence of low concentrations of hemoglobin, ferritin, and retinol for each group at baseline and 12 wk are presented in Table 4. After 12 wk of supplementation the prevalence of anemia (hemoglobin < 120 g/L) had decreased in the supplemented groups but the decrease was only significant ($P = 0.011$) in the group supplemented weekly with 60 mg Fe. The final prevalences of anemia in the three supplemented groups were similar and ranged from 5.7% to 7.8%. In the placebo group a small but nonsignificant increase in the prevalence occurred. The percentage of subjects with a ferritin concentration < 15 $\mu\text{g/L}$ was lowest in the group supplemented daily group but this prevalence did not differ significantly from the prevalence in the group supplemented weekly with 120 mg Fe

TABLE 2
Selected characteristics for all subjects at baseline and for subjects from whom a complete data set was obtained after 12 wk of supplementation

| | All subjects at baseline ($n = 363$) | Subjects with complete data ($n = 273$) |
|---|--|---|
| Physiological values | | |
| Age (y) | 16.7 \pm 1.0 ¹ | 16.8 \pm 0.9 |
| Weight (kg) | 47.7 \pm 6.6 | 47.9 \pm 6.9 |
| Height (cm) | 154.3 \pm 4.8 | 153.6 \pm 4.9 |
| Body mass index (kg/m ²) | 20.0 \pm 2.4 | 20.2 \pm 2.3 |
| Time since first menstruation (y) | 3.9 \pm 0.3 | 3.9 \pm 0.3 |
| Prevalence of low hematologic values | | |
| Hemoglobin < 120 g/L (%) | 17.4 | 17.2 |
| Ferritin < 15 $\mu\text{g/L}$ (%) ² | — | 30.4 |
| Retinol < 0.70 $\mu\text{mol/L}$ (%) ² | — | 30.0 |

¹ $\bar{x} \pm \text{SD}$.

² No ferritin and retinol analyses were done for the dropouts.

TABLE 3
Concentration of hemoglobin, ferritin, and retinol at baseline and after 8 and 12 wk of supplementation, and the respective changes compared with baseline

| Group | Absolute concentrations | | | Differences | |
|----------------------------|---------------------------------|---------------------------------|-----------------------------------|----------------------------|----------------------------|
| | Baseline | 8 wk | 12 wk | 8 - 0 wk | 12 - 0 wk |
| Hemoglobin (g/L) | | | | | |
| Daily (n = 64) | 127.6 ± 11.0 ¹ | 133.9 ± 9.9 | 130.9 ± 7.8 ² | 6.3 ± 9.9 ³ | 3.3 ± 8.8 ³ |
| Weekly | | | | | |
| Low iron content (n = 70) | 124.9 ± 10.6 | 131.0 ± 8.9 | 130.5 ± 7.8 ² | 6.1 ± 6.5 ³ | 5.6 ± 7.8 ³ |
| High iron content (n = 64) | 126.2 ± 10.6 | 131.2 ± 8.8 | 130.0 ± 7.7 ² | 5.0 ± 6.7 ³ | 3.7 ± 7.8 ³ |
| Placebo (n = 75) | 127.4 ± 10.1 | 126.0 ± 8.6 ⁴ | 124.6 ± 8.8 ^{2,4} | -1.4 ± 4.0 ^{4,5} | -2.8 ± 4.4 ^{3,4} |
| Ferritin (μg/L) | | | | | |
| Daily (n = 64) | 34.7 ± 22.0 (31.2) ⁶ | 59.5 ± 25.2 (56.8) | 61.9 ± 32.0 (57.9) | 24.8 ± 20.1 ³ | 27.2 ± 23.4 ³ |
| Weekly | | | | | |
| Low iron content (n = 70) | 27.5 ± 20.2 (24.0) | 37.3 ± 24.7 (33.8) ⁷ | 42.3 ± 22.0 (39.5) ^{2,7} | 9.8 ± 11.4 ^{3,7} | 14.8 ± 14.7 ^{3,7} |
| High iron content (n = 64) | 30.6 ± 25.6 (25.7) | 39.8 ± 24.4 (36.1) ⁷ | 44.8 ± 27.2 (40.9) ^{2,7} | 9.2 ± 14.5 ^{3,7} | 14.2 ± 16.4 ^{3,7} |
| Placebo (n = 75) | 32.1 ± 23.5 (27.9) | 29.0 ± 22.3 (25.0) ⁴ | 27.5 ± 18.6 (24.3) ⁴ | -3.1 ± 11.8 ^{4,5} | -4.6 ± 12.5 ^{3,4} |
| Retinol (μmol/L) | | | | | |
| Daily (n = 64) | 0.87 ± 0.22 | 1.12 ± 0.20 | 1.17 ± 0.18 | 0.25 ± 0.22 ³ | 0.30 ± 0.25 ³ |
| Weekly | | | | | |
| Low iron content (n = 70) | 0.85 ± 0.26 | 1.11 ± 0.23 | 1.12 ± 0.21 | 0.27 ± 0.27 ³ | 0.28 ± 0.25 ³ |
| High iron content (n = 64) | 0.81 ± 0.25 | 1.07 ± 0.21 | 1.15 ± 0.22 ² | 0.26 ± 0.18 ³ | 0.35 ± 0.24 ³ |
| Placebo (n = 75) | 0.84 ± 0.26 | 0.92 ± 0.18 ⁴ | 0.86 ± 0.16 ^{2,4} | 0.08 ± 0.20 ^{3,4} | 0.03 ± 0.25 ⁴ |

¹ $\bar{x} \pm SD$.

² Significantly different from corresponding value at 8 wk, $P < 0.001$.

^{3,5} Significant within-group change: ³ $P < 0.001$, ⁵ $P < 0.02$.

⁴ Significantly lower than groups supplemented weekly and daily, $P < 0.001$.

⁶ $\bar{x} \pm SD$; geometric means of square root transformation in parentheses.

⁷ Significantly lower than group supplemented daily, $P < 0.001$.

($P = 0.094$). Prevalence of low retinol values decreased significantly ($P < 0.01$) to 0% in the supplemented groups. This prevalence also decreased significantly ($P < 0.01$) from 30.7% to 9.3% in the placebo group although no significant changes occurred in the mean retinol concentration. Changes in the distribution of retinol concentrations for the three supplemented groups and for the placebo group ($n = 75$) are shown in **Figures 1** and **2**. Because the curves for the groups supplemented daily and weekly were similar they were pooled. Because of the treatment, the distribution curve of the supplemented groups shifted to the right. The distribution curve of the placebo group became more narrow without an important change in the median.

Six months after the end of the 12-wk supplementation period a blood sample was again obtained from a total of 172 subjects. The aim of this follow-up study was to investigate the iron stores, as indicated by ferritin concentrations, that re-

mained 6 mo after the supplementation had ended. Results are presented in **Table 5**. The ferritin concentration at baseline did not differ significantly between groups, and the concentration at baseline for this smaller group was similar to that of the whole group of 273 subjects. Six months after the supplementation had ended the placebo group had a lower ferritin concentration than the supplemented groups ($P = 0.003$). There were no significant differences between the groups supplemented daily and weekly. On the basis of changes between final and baseline ferritin concentrations, no between-group difference in treatment effect existed for the groups supplemented daily and weekly (interaction between treatment effect and treatment type, $P = 0.619$), contrary to the difference—which was nearly significant after 12 wk of supplementation—between the groups supplemented daily and weekly (interaction between treatment effect and treatment type, $P = 0.069$).

TABLE 4
Prevalence of low concentrations of hemoglobin, ferritin, and retinol at baseline and after 12 wk of supplementation

| Treatment | Hemoglobin < 120 g/L | | Ferritin < 15 μg/L | | Retinol < 0.70 μmol/L | |
|----------------------------|----------------------|-------------------|--------------------|-------------------|-----------------------|------------------|
| | 0 wk | 12 wk | 0 wk | 12 wk | 0 wk | 12 wk |
| Daily (n = 64) | 15.6 | 7.8 | 21.9 | 1.6 ¹ | 21.9 | 0 ¹ |
| Weekly | | | | | | |
| Low iron content (n = 70) | 20.0 | 5.7 ¹ | 32.9 | 5.7 ¹ | 31.4 | 0 ¹ |
| High iron content (n = 64) | 15.6 | 7.8 | 35.9 | 7.8 ¹ | 35.9 | 0 ¹ |
| Placebo (n = 75) | 17.3 | 21.3 ² | 30.7 | 36.0 ³ | 30.7 | 9.3 ³ |

¹ Significant within-group decrease in prevalence, $P < 0.01$.

^{2,3} Significantly different from supplemented groups: ² $P < 0.05$, ³ $P < 0.01$.

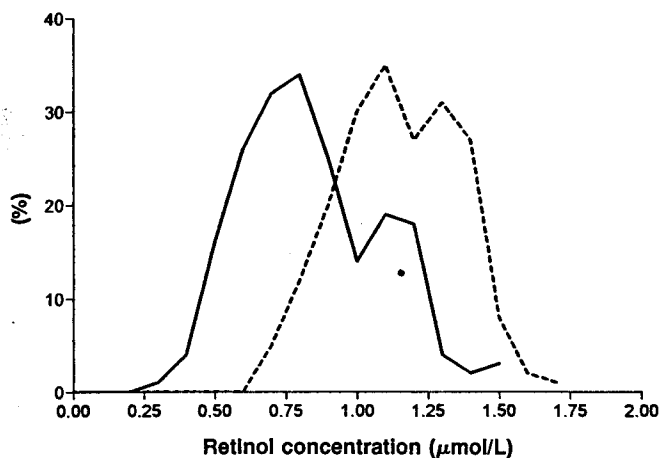


FIGURE 1. Distribution of retinol concentration of the micronutrient-supplemented groups combined ($n = 198$) at baseline (—) and after 12 wk of supplementation (- - -).

Among the group of 172 subjects at baseline, the prevalence of low ferritin concentrations ($< 15 \mu\text{g/L}$) was 18.9%, 28.9%, 35.0%, and 32.0% for the group supplemented daily, weekly with 60 mg Fe, weekly with 120 mg Fe, and with placebo, respectively. Six months after the end of the supplementation the corresponding prevalences were 10.8%, 15.6%, 17.5%, and 44.0%, respectively.

Tablet intake was supervised during the first 8 wk of the supplementation period to ensure that all tablets were taken. During weeks 9–12, tablets were given on a take-home basis. Checking tablet intake during weeks 9–12 indicated that subjects from each of the three supplemented groups took on average about two of the four tablets prescribed for Fridays. For the remaining days of the week there was no difference between the supplemented groups. On average, subjects from each group took 7–8 of the prescribed 16 tablets (tablets for Monday–Thursday). Total intake for the supplemented groups (including placebo for the groups supplemented weekly) was 9.4 tablets on average, whereas for the placebo group the intake was higher at 10.8 tablets ($P < 0.05$).

Subjects were questioned about side effects that occurred during weeks 10 and 11 of the supplementation period, and the

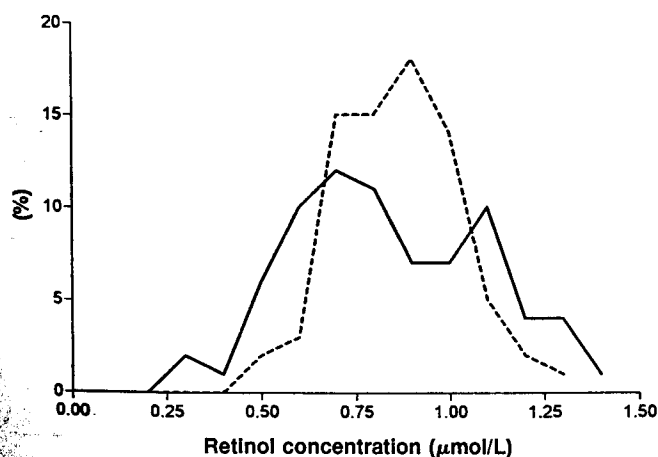


FIGURE 2. Distribution of retinol concentration of the placebo group ($n = 75$) at baseline (—) and after 12 wk of supplementation (- - -).

percentage of subjects experiencing a certain side effect during that period are shown in Table 6. Absence of side effects was most frequently reported in the placebo group, followed by the group supplemented weekly with 60 mg Fe. Nausea, vomiting, sleepiness, and increased appetite were significantly associated with frequency and amount of iron intake. The percentage of subjects who mentioned nausea was lower among individuals receiving 60 mg Fe weekly than among those of the two other supplemented groups ($P < 0.05$). The frequency of vomiting was higher among those receiving daily supplementation than among members of the other three groups ($P < 0.05$).

DISCUSSION

Iron status of adolescent girls is likely to be an important determinant of anemia during an eventual future pregnancy. This is especially the case in the majority of developing countries where the average age of a first pregnancy is relatively low. A 1994 population survey in Indonesia showed that the median age at first birth was ≈ 20 y and that 33% of women aged 20–24 y at the time of the survey had their first child before they were 19 y age (21).

The group of adolescent girls in this investigation was relatively privileged because they still went to school. In Indonesia, the majority of 17-y-old girls would probably already be working or be married. Considering their privileged position, their prevalence of iron and vitamin A deficiency was quite high. The high prevalence (30%) of subjects with low retinol values, and the correlation between retinol and hemoglobin concentrations, retrospectively supported the approach of using combined iron-vitamin A supplementation, as had been suggested earlier for combating anemia (8, 9).

Several aspects of supplementation were investigated in this study—the composition of supplements: 60 mg compared with 120 mg Fe; the frequency of supplementation: weekly compared with daily; the duration of supplementation: 8 compared with 12 wk; and the effect of supplementation on iron status 6 mo after the last dose. Furthermore, side effects were recorded.

Iron and vitamin A status of the groups supplemented daily and weekly improved significantly compared with that of the placebo group, indicating the beneficial effect of the supplementation. In the placebo group, no improvements occurred in iron status, but the prevalence of low retinol concentrations was reduced from 30.7% to 9.3%. This reduction is probably due to a combined effect of deworming and a regression to the mean. The positive effect of deworming on vitamin A status was reported previously in a study among Brazilian and Indonesian children (22, 23).

Between the two groups supplemented weekly, no difference in treatment effect resulted from 60 compared with 120 mg Fe and a vitamin A–iron ratio of 100 compared with 50. The group supplemented with 120 mg Fe, however, did complain more frequently about side effects, hence, the weekly supplement containing 60 mg Fe gave better overall results than the one containing 120 mg Fe.

Comparing the groups supplemented daily and weekly, there was no difference in treatment effect on hemoglobin and retinol concentrations. However, the increase in ferritin concentration of the subjects supplemented daily was 12–13- $\mu\text{g/L}$ higher ($P < 0.001$) than that of the subjects supplemented weekly.

TABLE 5
Ferritin concentration at baseline, after 12 wk of supplementation, and 6 mo after the end of supplementation

| Treatment | Absolute concentrations | | | Difference: 6 mo - baseline |
|------------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|
| | Baseline | 12 wk | 6 mo after | |
| Daily (<i>n</i> = 37) | 34.7 ± 20.7 (31.5) ¹ | 63.4 ± 34.6 (59.0) | 40.6 ± 23.9 (37.1) | 5.9 ± 20.6 ² |
| Weekly | | | | |
| Low iron content (<i>n</i> = 45) | 27.3 ± 19.8 (23.8) | 41.8 ± 20.4 (39.4) ³ | 34.5 ± 19.9 (31.9) | 7.2 ± 13.1 ⁴ |
| High iron content (<i>n</i> = 40) | 29.2 ± 22.2 (25.3) | 44.9 ± 27.8 (41.1) ³ | 35.5 ± 23.9 (31.6) | 6.3 ± 11.8 ⁴ |
| Placebo (<i>n</i> = 50) | 29.9 ± 22.1 (26.2) | 27.4 ± 18.7 (24.3) ⁵ | 25.1 ± 18.4 (22.1) ⁶ | -4.8 ± 14.3 ^{5,7} |

¹ $\bar{x} \pm SD$; geometric means of square root transformations in parentheses.

² $\bar{x} \pm SD$.

³ Significantly lower than group supplemented daily, $P < 0.001$.

^{4,7} Significant within-group change between baseline and 6 mo postsupplementation: ⁴ $P < 0.01$, ⁷ $P < 0.02$.

^{5,6} Significantly lower than groups supplemented weekly and daily: ⁵ $P < 0.001$, ⁶ $P < 0.01$.

The ferritin concentration of the groups supplemented weekly was $\approx 40 \mu\text{g/L}$ after 12 wk, and $\approx 7\%$ of the subjects still had a concentration $< 15 \mu\text{g/L}$. Although lower than in the group supplemented daily, the average ferritin concentration reached by the subjects supplemented weekly after 12 wk can be considered quite satisfactory, especially compared with ferritin values reported for Western populations. Apparently healthy 12-14-y-old British school girls had an average ferritin concentration of $30.4 \mu\text{g/L}$, with 19.8% having a ferritin value $< 20 \mu\text{g/L}$ (24). Among a United States population of 15-19-y-old females, 14.2% had a ferritin concentration $< 12 \mu\text{g/L}$ (25). It should also be noted that subjects supplemented daily complained more about side effects, specifically vomiting and nausea, than did subjects supplemented weekly with 60 mg Fe.

The real effect of supplementation from 8 to 12 wk was difficult to estimate because the assessed tablet intake for this period was $\approx 50\%$ of the targeted intake. Despite the reduced intake, a further increase in iron stores occurred in the groups supplemented weekly, as indicated by a rise in ferritin concentration of $5 \mu\text{g/L}$. In the group supplemented daily, no significant additional increase in ferritin occurred even though the absolute amount of ingested iron was more than twice that among the groups receiving the supplement weekly. The lack of significant increase in ferritin concentration the group supplemented daily compares well with results from a study among young healthy Swedish women, which suggested that the net absorption of iron from diets with medium to high

bioavailability becomes zero when ferritin concentrations range from 40 to $60 \mu\text{g/L}$ (26).

Supplementation of adolescents with the objective to build iron stores will only be efficient if the supplementation effect remains during a prolonged period of time. Six months after the end of the 12-wk supplementation the ferritin concentration of the three supplemented groups was still $6 \mu\text{g/L}$ higher ($P < 0.001$) than initial values, and the prevalence of low ferritin concentrations ($< 15 \mu\text{g/L}$) was about half that before the intervention. Six months after the end of supplementation the previous difference in ferritin concentration between daily and weekly supplementation was not significant anymore because the decrease in ferritin concentration over the 6-mo period was greater in the group supplemented daily than in the group supplemented weekly. This greater decrease in the group supplemented daily may be due to a low rate of absorption of iron from food, which occurs when body stores are high (26). Therefore, the effect on ferritin concentration of daily compared with weekly supplementation was similar several months after the end of supplementation.

The weekly supplement with 60 mg Fe and $6000 \mu\text{g}$ retinol was most efficient in improving hemoglobin and retinol status and gave good results in building iron stores when used over a period of 12 wk. The vitamin A content of the weekly supplements was $6000 \mu\text{g}$, which is ≈ 1.7 times the weekly safe intake recommended by the Food and Agriculture Organization/World Health Organization (27). In view of the lack of further improvement in

TABLE 6
Percentage of subjects who reported side effects related to the supplementation during weeks 10 and 11 of the study period


| Side effect | Treatment group | | | |
|--------------------|------------------------|-----------------------------------|------------------------------------|--------------------------|
| | Daily (<i>n</i> = 64) | Weekly | | Placebo (<i>n</i> = 75) |
| | | Low iron content (<i>n</i> = 70) | High iron content (<i>n</i> = 64) | |
| | | % | | |
| Nausea | 20.3 | 5.7 ¹ | 25.0 | 1.3 ² |
| Vomiting | 12.5 ³ | 0.0 | 3.1 | 0.0 |
| Diarrhea | 0.0 | 0.0 | 3.1 | 5.4 |
| Sleepiness | 11.0 | 15.7 | 21.9 | 1.3 ² |
| Increased appetite | 10.9 | 8.6 | 6.3 | 0.0 ² |
| No side effects | 45.3 | 70.0 ¹ | 40.6 | 92.0 ² |

¹ Significantly different from group supplemented daily and group supplemented weekly with the high amount of iron, $P < 0.05$.

² Significantly different from micronutrient-supplemented groups, $P < 0.05$.

³ Significantly different from other groups, $P < 0.05$.

retinol concentration during weeks 9–12 of the supplementation and the additional costs, a reduction of the vitamin A content of the supplement to $\approx 4000 \mu\text{g}$ retinol should be considered. Therefore, under conditions similar to the situation at baseline in the present study, a weekly supplement with 60 mg Fe and 4000 μg retinol will probably give good results in the improvement of iron and vitamin A status and iron stores will be retained for ≥ 6 mo. Anthelmintic treatment may contribute significantly to the improvement of vitamin A status when a large part of the population is infested with helminths.

The first 8 wk of the supplementation were supervised, which ensured complete tablet intake. When tablets were given on a take-home basis the intake dropped to $\approx 50\%$ of the prescribed intake. Therefore, it is important to investigate the effectiveness of weekly supplementation of adolescents under programmatic conditions before making any final decisions about larger-scale applications. Topics to be investigated further include types of distribution systems, factors that influence compliance with tablet intake such as knowledge and attitude toward micronutrients, and communication strategies. 

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